

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0022] with the following amended paragraph:

[0022] FIG. 2 is a depiction of a preferred bifunctional agent (R1 = SEQ ID NO:1; R2 = SEQ ID NO:2; and R3 = SEQ ID NO:3).

Please replace paragraph [0042] with the following amended paragraph:

[0042] In a preferred embodiment, the targeting moiety is a substrate or inhibitor for cathepsin K. Cathepsin K is also an elastolytic cysteine protease, and is considered to be the most potent mammalian elastase, and also has collagenolytic activity. Cathepsin K is considered unique among mammalian proteinases in that its collagenolytic activity does not depend on the destabilization of the triple helix of collagen in contrast to other cysteine proteases and that it cleaves native molecules at more sites than does interstitial collagenase. Thus, cathepsin K can degrade completely the insoluble collagen of adult cortical bone in the absence of other proteases. It is highly expressed in osteoclasts. It plays an important role in bone resorption and is essential for normal bone growth and remodeling. It has been implicated in osteoporosis, pycnodysostosis, bone cancer as well as breast cancer. It is interesting to note that, breast cancer commonly metastasizes to bone, and cathepsin K was initially identified as related to breast cancer by its presence in breast cancer cells that had spread to and invaded bone. Its substrates include, but are not limited to, elastin and collagen, and its inhibitors include, but are not limited to, Cbz-Gly-Arg-AMC; Cbz-Arg-Arg-AMC; Cbz-Gly-Gly-Arg-AMC; Cbz-Ala-Lys-Arg-AMC; Cbz-Ala-Arg-Arg-AMC; Cbz-d-Phe-Arg-AMC; Boc-Leu-Gly-Arg-AMC; H-Gly-Arg-AMC; H-Ala-Arg-AMC; Cbz-Leu-Leu-Leu-AMC; Cbz-Leu-Leu-AMC; Cbz-Phe-Gly-AMC; Cbz-Gly-Gly-Leu-AMC; Suc-Ala-Ala-Val-AMC; Cbz-Gly-Ala-Met-AMC; E-64; Leupeptin (Ac-Leu-Leu-Arg-CHO); N-acetyl-Leu-Leu-methional; Ac-Leu-Leu-Met-CHO; Ac-Leu-Val-Lys-CHO; Ac-Leu-Leu-Nle-CHO; Cbz-Lys-Leu-Leu-CHO; Cbz-Leu-Leu-Leu-CHO; Cbz-Arg-Leu-Leu-CHO; Series of 1,3-bis(acylamino)-2-propanones; series of 1,3-diamino ketones; and a series of 1,5-diacylcarbohydrazides. Suitable cathepsin K substrates include, but are not limited to, Cbz-Leu-Arg-AMC; Cbz-Val-Arg-

AMC; Cbz-Phe-Arg-AMC; Cbz-Leu-Leu-Arg-AMC; Tos Gly-Pro-Arg-AMC; Bz-; Phe-Val-Arg-AMC; H-Pro-Phe-Arg-AMC; Cbz-Val-Val-Arg-AMC; Boc-Val-ProArg-AMC; Cbz-Glu-Arg-AMC; Bz-Arg-AMC; Ac-Phe-Arg-AMC; Boc-Val-Leu-Lys-AMC; Suc-Leu-TyrAMC; Boc-Ala-Gly-Pro-Arg-AMC (SEQ ID NO:4); Cbz-Gly-Pro-Arg-AMC; Z-Leu-Arg-4-methoxy-b-naphthylamide (where Cbz=benzyloxycarbonyl and AMC=aminomethylcoumarin); diaminopropanones, diacylhydrazine and cystatin C. See Bossard, M. J. et al., J. Biol. Chem. 271, 12517-12524 (1996); Aibe, K. et al., Biol. Pharm. Bull. 19,1026-1031 (1996); Votta, B. J. et al. J. Bone Miner. Res. 12, 13961406 (1997); Yamshita, D. S. et al. J. Am. Chem. Soc. 119,11351-11352 (1997); DesJarlais, R. L. et al. J. Am. Chem. Soc. 120, 9114-9115 (1998); Marquis, R. W. et al. J. Med. Chem. 41, 3563-3567 (1998); Thompson et al., J. Med. Chem. 41, 3923-3927 (1998); Thompson et al., Bioorg. Med. Chem. 7, 599605 (1999); Kamiya, T. et al. J. Biochem. (Tokyo) 123, 752-759 (1998), Shi et al, J. Clin. Invest. 104:1191 (1999); and Sukhova et al., J. Clin. Invest. 102:576 (1998), all of which are expressly incorporated by reference, and all of which can be used as targeting moieties.

Please replace paragraph [0046] with the following amended paragraph:

[0046] In a preferred embodiment, the targeting moiety is a substrate or inhibitor for a matrix metalloproteinase (MMP), of which a variety are known. In general, known inhibitors of MMPs are chemically modified tetracyclines (CMTs), a number of which are listed below. The CMTs include, but are not limited to, 4-dimethylamino-TC (also known as CMT-1); tetracycinonitrile (CMT-2); 6-demethyl, 6-deoxy, 4-dedimethylamino-TC (CMT-3); 7-chloro, 4-dedimethylamino-TC (CMT-4); 4-hydroxy, 4-dedimethylamino-TC (CMT-6); 12a-deoxy, 5-hydroxy-4-dedimethylamino-TC (CMT-7); 6a-deoxy, 5 hydroxy-4-dedimethylamino-TC (CMT-8); 12a, 4a-anhydro; 4-dedimethylamino-TC (CMT-9); 7-dimethylamino, 4-dedimethylamino-TC (CMT-10). In addition to the CMTs, other known inhibitors of MMPs include the tissue inhibitors of MPs-1 and MPs-2 (TIMP-1 and TIMP-2, respectively) and minocycline (Min) and doxycycline (Dox). Suitable targeting moieties comprising peptide substrates for

MMPs include the peptide sequence Pro-Met-Ala-Leu-Trp-Met-Arg (SEQ ID NO:5) (Netzel-Arnett, S., et al., 1993, Biochem., 32: 6427-6432). Recognition of the peptide sequence by an MMP can result in cleavage of the peptide sequence Pro-Met-Ala-Leu-Trp-Met-Arg (SEQ ID NO:5) to yield two peptide fragments: -Pro-Met-Ala- and -Leu-Trp-Met-Arg (SEQ ID NO:6). Preferred peptide substrates include -Ala-Leu-. There are a number of other MMP inhibitors and substrates that can be used as targeting moieties. The substrates are particularly useful as cancer cleavage sites with the use of coordination site barriers. These MMP inhibitors and substrates include, but are not limited to, 1,10-phenanthroline; CT 1847; AG3319, AG3340 (also called Prinomastat), AG3287, AG3293, AG3294, AG3296; 2-mercaptoacetyl L-phenyl-alanyl-L-leucin- e; HSCH₂ CH[CH₂CH(CH₃)₂]CO-Phe-Ala-NH₂; OPB-3206; Furin Inhibitor; 3,4-dihydro-1-oxo-1,2,3,-benzotriazine-3-(3-tetrahydrofuranyl)carbonate (IW-1); 1,2-dihydro-3,6dioxo-2-phenyl-pyridazine-1-methylcarbonate (LW-2); 3,4-dihydro-1-oxo-1,2,3,-benzotriazine-3-(2methoxy) ethylcarbonate (LW-3); 1,2-dihydro-2-ethoxycarbonyl-(1-oxo-isochinolin-5-- yl) ethylcarbonate (LW-4); 1(2H)-phtalazinone-2-(4-methoxyphenyl) carbonate (LW-5); N-[2(R)-2-(hydroxamido carbonylmethyl)-4-methyl pentanoyl]-L-tryptophane methylamide also called GM6001, Galardin and ilomastat; BAY 12-9566; Neovastat (AE-941); BB-1101; G1129471; Ph(CH₂NH-D-RrevCO-CH₂CH₂-D)₂ also called FC-336; Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ (SEQ ID NO:7) (cleavage occurs between Gly and Leu); DNP-Pro-Leu-Gly-Ile-Ala-Gly-Arg-NH₂OOOH (SEQ ID NO:8) (cleavage occurs between Gly and Leu); arboxymethyl transferrin (Cm-Tf); (7-methoxycoumarin-4-yl)acetyl-PLGP-[3-(2,4-dinitrop- henyl)-L-2,3 diaminopropionyl]-AR-NH₂ (SEQ ID NO:9); (7-methoxycoumarin-4-yl)acetyl-PLAQ- AV-[3-(2,4-dinitrophenyl)-L-2,3 diaminopropionyl]-RSSSR-NH₂ (SEQ ID NO:10); Ac-PLG-[2-mercapto-4-methylpentanoyl]-LG-OEt; Peptide I: GPLGLRSW (SEQ ID NO:11); and Peptide II: GPLPLRSW (SEQ ID NO:12). See generally, Greenwald, R. A. et al. In vitro sensitivity of the three mammalian collagenases to tetracycline inhibition: relationship to bone and cartilage degradation. Bone 22, 33-38 (1998); Kolb, S. A. et al. Matrix metalloproteinases

and tissue inhibitors of metalloproteinases in viral meningitis: upregulation of MMP-9 and TIMP-1 in cerebrospinal fluid. *J. Neuroimmunol.* 84, 143-150 (1998); Charoenrat, P. et al. Overexpression of epidermal growth factor receptor in human head and neck squamous carcinoma cell lines correlates with matrix metalloproteinase-9 expression and in vitro invasion. *Int. J. Cancer* 86, 307-317 (2000); Uzui, H., Lee, J. D., Shimizu, H., Tsutani, H. & Ueda, T. The role of protein-tyrosine phosphorylation and gelatinase production in the migration and proliferation of smooth muscle cells. *Atherosclerosis* 149, 51-59 (2000); Montesano, R., Soriano, J. V., Hosseini, G., Pepper, M. S. & Schramek, H. Constitutively active mitogen-activated protein kinase kinase MEK1 disrupts morphogenesis and induces an invasive phenotype in Madin-Darby canine kidney epithelial cells. *Cell Growth Differ.* 10, 317-332 (1999); Yip, D., Ahmad, A., Karapetis, C. S., Hawkins, C. A. & Harper, P. G. Matrix metalloproteinase inhibitors: applications in oncology. *Invest New Drugs* 17, 387-399 (1999); Price, A. et al. Marked inhibition of tumor growth in a malignant glioma tumor model by a novel synthetic matrix metalloproteinase inhibitor AG3340. *Clin. Cancer Res.* 5, 845-854 (1999); Santos, O., McDermott, C. D., Daniels, R. G. & Appelt, K. Rodent pharmacokinetic and anti-tumor efficacy studies with a series of synthetic inhibitors of matrix metalloproteinases. *Clin. Exp. Metastasis* 15, 499-508 (1997); Barletta, J. P. et al. Inhibition of pseudomonal ulceration in rabbit corneas by a synthetic matrix metalloproteinase inhibitor. *Invest Ophthalmol. Vis. Sci.* 37, 20-28 (1996); Maquoi, E. et al. Inhibition of matrix metalloproteinase 2 maturation and HT1080 invasiveness by a synthetic furin inhibitor. *FEBS Lett.* 424, 262-266 (1998); Makela, M. et al. Matrix metalloproteinase 2 (gelatinase A) is related to migration of keratinocytes. *Exp. Cell Res.* 251, 67-78 (1999); Hao, J. L. et al. Effect of galardin on collagen degradation by *Pseudomonas aeruginosa*. *Exp. Eye Res.* 69, 595-601 (1999); Hao, J. L. et al. Galardin inhibits collagen degradation by rabbit keratocytes by inhibiting the activation of pro-matrix metalloproteinases. *Exp. Eye Res.* 68, 565-572 (1999); Wallace, G. R. et al. The matrix metalloproteinase inhibitor BB-1101 prevents experimental autoimmune uveoretinitis (EAU). *Clin. Exp. Immunol.* 118, 364-370 (1999); Maquoi, E. et al.

Membrane type 1 matrix metalloproteinase-associated degradation of tissue inhibitor of metalloproteinase 2 in human tumor cell lines: *J. Biol. Chem.* 275, 11368-11378 (2000); Ikeda, T. et al. Anti-invasive activity of synthetic serine protease inhibitors and its combined effect with a matrix metalloproteinase inhibitor. *Anticancer Res.* 18, 4259-4265 (1998); Schultz, S. et al. Treatment of alkali-injured rabbit corneas with a synthetic inhibitor of matrix metalloproteinases. *Invest Ophthalmol. Vis. Sci.* 33, 3325-3331 (1992); Buchardt, J. et al. Phosphinic Peptide Matrix Metalloproteinase-9 Inhibitors by Solid-Phase Synthesis Using a Building Block Approach. *Chem. Eur. J.* 5, 2877-2884 (2000); Dahlberg, L. et al. Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). *Arthritis Rheum.* 43, 673-682 (2000); Lombard, M. A. et al. Synthetic matrix metalloproteinase inhibitors and tissue inhibitor of metalloproteinase (TIMP)-2, but not TIMP-1, inhibit shedding of tumor necrosis factor-alpha receptors in a human colon adenocarcinoma (Colo 205) cell line. *Cancer Res.* 58, 4001-4007 (1998); Lein, M. et al. Synthetic inhibitor of matrix metalloproteinases (batimastat) reduces prostate cancer growth in an orthotopic rat model. *Prostate* 43, 77-82 (2000); Brown, P. D. Matrix metalloproteinase inhibitors in the treatment of cancer. *Med. Oncol.* 14, 1-10 (1997); Garbett, E. A., Reed, M. W. & Brown, N. J. Proteolysis in colorectal cancer. *Mol. Pathol.* 52, 140-145 (1999); Itoh, M. et al. Purification and refolding of recombinant human proMMP-7 (pro-matrilysin) expressed in *Escherichia coli* and its characterization. *J. Biochem. (Tokyo)* 119, 667673 (1996); Wang, Y., Johnson, A. R., Ye, Q. Z. & Dyer, R. D. Catalytic activities and substrate specificity of the human membrane type 4 matrix metalloproteinase catalytic domain. *J. Biol. Chem.* 274, 3304333049 (1999); Ohkubo, S. et al. Identification of substrate sequences for membrane type-1 matrix metalloproteinase using bacteriophage peptide display library. *Biochem. Biophys. Res. Commun.* 266, 308-313 (1999), all of which are expressly incorporated by reference, and all of which can be used as targeting moieties.

Please replace paragraph [0058] with the following amended paragraph:

[0058] In a preferred embodiment, the targeting moiety is involved in angiogenesis.

There are a wide variety of moieties known to be involved in angiogenesis, including, but not limited to, vascular endothelial growth factors (VEGF; including VEGF-A, VEGF-B, VEGF-C and VEGF-D), FGF-1 (aFGF), FGF-2 (bFGF), FGF-3, FGF-4, hepatocyte growth factor (HGF, scatter factor), thymidine phosphorylase, angiogenin, IL-8, TNF- α , leptin, transforming growth factors (TGF- α , TGF- β), platelet-derived growth factor, proliferin, and granulocyte colony stimulating factor (G-CSF). Known angiogenesis inhibitors include, but are not limited to, platelet factor 4, thrombospondin-1, interferons (IFN- α , IFN- β , IFN- γ), IL-1, IL-2, vascular endothelial growth inhibitor (VEGI), 2-methoxyestradiol, tissue inhibitors of MMPs (TIMPs), proliferin related protein, angiostatin, endostatin, amion terminal fragment of u-PA (ATF), thalidomide, TNP-470/AGM-1470, carboxyamidotriazole, maspin, AG3340, marimastat, BAY9566, CSG-27023A, gly-arg-gly-asp-ser (GRGDS SEQ ID NO:13), tyr-ile-gly-ser-arg (YIGSR SEQ ID NO:14) and ser-ile-lys-val-ala-val (SIKVAV SEQ ID NO:15). See van Hinsbergh et al, Annals of Oncology 10 Supp. 4:60 (1999) and references therein; Li et al., Human Gene Therapy 10(18):3045 (1999); Duenas et al., Investigative Ophthalmology, 1999; Bauer et al., J. Pharmacology & Experimental Therapeutics 292(1):31 (2000); Zhang et al., Nature Medicine 6(2):196 (2000); Sipose et al., Annal of the New York Academy of Sciences 732:263 (1994 and references therein); Niresia et al, Am. J. Pathology 138(4):829 (1991); Yamamura et al., Seminars in Cancer Biology 4(4):259 (1993). Thus moieties which bind to these factors are useful as targeting moieties in the present invention.

Please replace paragraph [0074] with the following amended paragraph:

[0074] In a preferred embodiment, the targeting moiety is a nuclear localization signal (NLS). NLSs are generally short, positively charged (basic) domains that serve to direct the moiety to which they are attached to the cell's nucleus. Numerous NLS amino acid sequences have been reported including single basic NLS's such as

that of the SV40 (monkey virus) large T Antigen (Pro Lys Lys Lys Arg Lys Val; SEQ ID NO:16), Kalderon (1984), et al., Cell, 39:499-509; the human retinoic acid receptor-1 nuclear localization signal (ARRRRP; SEQ ID NO:17); NF[[?]]kappaB p50 (EEVQRKRQKL; SEQ ID NO:18; Ghosh et al., Cell 62:1019 (1990); NF[[?]]kappaB p65 (EEKRKRTYE; SEQ ID NO:19; Nolan et al., Cell 64:961 (1991); and others (see for example Boulikas, J. Cell. Biochem. 55(1):32-58 (1994), hereby incorporated by reference) and double basic NLS's exemplified by that of the Xenopus (African clawed toad) protein, nucleoplasmin (Ala Val Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys Leu Asp; SEQ ID NO:20), Dingwall, et al., Cell, 30:449-458, 1982 and Dingwall, et al., J. Cell Biol., 107:641-849; 1988). Numerous localization studies have demonstrated that NLSs incorporated in synthetic peptides or grafted onto reporter proteins not normally targeted to the cell nucleus cause these peptides and reporter proteins to be concentrated in the nucleus. See, for example, Dingwall, and Laskey, Ann, Rev. Cell Biol., 2:367-390, 1986; Bonnerot, et al., Proc. Natl. Acad. Sci. USA, 84:6795-6799, 1987; Galileo, et al., Proc. Natl. Acad. Sci. USA, 87:458-462, 1990.

Please replace paragraph [0098] with the following amended paragraph:

[0098] Thus, a preferred embodiment is shown in FIGS. 2 and 3, which depict dyads (bifunctional agents), comprising any or all of: (1) a one photon PDT moiety with a targeting moiety (shown in the figure as ~~somatostatin-14~~ Somatostatin-14 SEQ ID NO:1, octreotate SEQ ID NO:2 or a derivative, but any of the above targeting moieties are included, with peptides being particularly preferred); (2), a two photon PDT moiety (2PM) with a targeting moiety; (3) a one photon PDT moiety, a targeting moiety and an imaging moiety; or (4) a two photon PDT moiety, a targeting moiety and an imaging moiety.